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Characterisation of gut microbiota of obesity and type 2 diabetes in a rodent model

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ABSTRACT

Various studies have suggested that the gut microbiome interacts with the host and may have a significant role in the aetiology of obesity and Type 2 Diabetes (T2D). It was hypothesised that bacterial communities in obesity and T2D differ from control and compromise normal interactions between host and microbiota. Obesity and T2D were developed in rats by feeding a high-fat diet or a high-fat diet plus a single low-dose streptozotocin administration, respectively. The microbiome profiles and their metabolic potentials were established by metagenomic 16S rRNA sequencing and bioinformatics. Taxonomy and predicted metabolism-related genes in obesity and T2D were markedly

different from controls and indeed from each other. Diversity was reduced in T2D but not in Obese rats. Factors likely to compromise host intestinal, barrier integrity were found in Obese and T2D rats including predicted, decreased bacterial butyrate production. Capacity to increase energy extraction via ABC-transporters and carbohydrate metabolism were enhanced in Obese and T2D rats. T2D was characterized by increased proinflammatory molecules. While obesity and T2D show distinct differences, results suggest that in both conditions *Bacteroides* and *Blautia* species were increased indicating a possible mechanistic link.

Keywords; butyrate, inflammatory molecules, microbiome, obesity, Type 2 diabetes

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INTRODUCTION

The worldwide incidence of obesity and diabetes mellitus (DM) has significantly increased in recent years and efforts to address this silently-killing disease are urgently required. During the last decade, several studies have focused on the role of the gut microbiota in maintenance of gut health and wellbeing. It has been proposed that an altered microbial-community might result in greater levels of energy being harvested from food, particularly from a high fat diet, and several mechanisms facilitate metabolic disorders, particularly Type 2 Diabetes (T2D) [1, 2]. These changes include the production of short chain fatty acids (SCFAs) and lipopolysaccharide (LPS) which cause chronic low-grade-inflammation [3]. Microbiome-profiling has been developed to determine the metagenomic structure of bacterial communities based on analysis of 16S rRNA sequences with software such as Quantitative Insights Into Microbial Ecology (QIIME) [4]. Recently, Phylogenetic Investigation of Communities by Reconstruction of Unobserved States (PICRUSt) has been developed to provide a view of metagenome function from 16S rRNA metagenomics or from full genomes [5].

Rat models have significantly contributed to the study of the function and role of microbiota in the gastrointestinal tract and its association with diseases such as metabolic disorder and obesity [6, 7], type 1 diabetes [8-10] and other complex diseases [11]. A model of T2D in rats has been introduced by Reed et al [12] and subsequently refined by various investigators (reviewed in [13]). The rats are maintained on a high-fat diet to produce obesity, hyperinsulinaemia, glucose intolerance and insulin resistance. Subsequent administration of a low dose of streptozotocin results in a reduction of pancreatic β-cell function. We hypothesise that the gut microbiome varies with phenotype and the aim of the present study was to characterize the composition of gut microbiota using 16S rRNA sequencing in two rat models: a model of obesity, induced by feeding a high-fat diet; and a model of T2D induced by high fat diet and a single, low-dose injection of streptozotocin (STZ). The identification of bacteria that contribute to protection of host and those that cause harm will potentially open the door to novel therapies for obesity and T2D and will provide clues to links between the two metabolic diseases.

MATERIALS AND METHODS

Animal maintenance and treatment

In-house-bred, male Wistar rats (age 10-12 weeks, 250-350 g, n=24) were maintained at room temperature (25°C) and 12/12-hour light-dark cycle and housed in standard cages (3 rats/ cage). At the beginning of the procedure (week 1), the rats were divided randomly into four groups, with 6 animals in each group and treated for a period of 12 weeks as described below. After 12 weeks, faecal pellets were collected from each group early in the morning (7:30 am) from animals housed individually over-night and immediately stored at -80°C.

Control group: (Normal Diet Vehicle). Rats were fed a normal diet (RM1, Rat and Mouse No. 1 Maintenance Diet; SDS, UK) containing crude fat of 2.7% by weight. Energy provision by component is: 13% calories from fat, 22% from protein and 65 % from carbohydrate with Gross Energy 14.72 MJ/kg (Summary composition in Table S1; full analysis at http://www.sdsdiets.com/pdfs/RM1P-E-FG.pdf). They received a single intraperitoneal (I/P) injection of citrate buffer (pH 4.4) in a volume of 1 mL kg^{-1}at 4 weeks. Rats were maintained on the same diet for another 8 weeks.

STZ-alone group: (Normal Diet Streptozotocin). Rats were fed a normal RM1 diet and received a single I/P injection of streptozotocin (STZ) in citrate buffer at 30 mg kg^{-1}at 4 weeks, and maintained on same diet for another 8 weeks.

Obese group (Ob): (High-Fat Diet Vehicle). Rats were fed a high fat diet (HFD; product code 821424, SDS, UK) containing crude fat of 22% by weight. Energy provision by component is: 45% calories from fat, 18% from protein and 37% from carbohydrate with Gross Energy 19.67 MJ/kg (Table S1). They also received a single I/P injection of citrate buffer (pH 4.4) in a dose of 1 mL kg^{-1} at 4 weeks. Rats were maintained on HFD for another 8 weeks.

Diabetic group (T2D): (High-Fat Diet STZ). Rats these rats were fed a HFD and injected I/P with a single I/P injection of STZ at 30 mg kg^{-1} at 4 weeks. Rats were maintained on HFD for another 8 weeks.

Animal weights and blood glucose were measured weekly. Blood glucose levels were measured using a glucometer (Accu-Check Aviva System; Roche Diagnosis, USA). An insulin tolerance test (ITT) was carried out in Control (Control; n=3) and Diabetic (T2D; n=3) groups 10 weeks after-the vehicle or STZ injection. Rats were fasted for 6 hours then received an I/P injection of bovine insulin (1U kg^{-1}; Sigma, UK). Blood samples were collected from the tail tip just before insulin administration (time 0) and at 30, 60, and 120 minutes after glucose/insulin injection for measurement of blood glucose concentration using the glucometer.

Bacterial DNA extraction from faecal pellets and Illumina MiSeq sequencing

Genomic DNA was isolated, within one day of faecal collection using the QIAamp DNA Stool Mini Kit (QIAGEN Manchester Limited (UK)) following the manufacturer's protocol. Separate isolations were made from three individual pellets/animal with material being taken from three separate locations on each pellet (180-220 mg for each animal), then placed in a 2 mL Lysing Matrix E (4 mm glass beads) microcentrifuge tubes (MP Biochemicals, Strasbourg). Tube contents were thoroughly homogenized in ASL Buffer using a FastPrep®-24 Instrument (MP Biomedicals, UK) at 4.5 M $second^{-1}$ for 30 seconds. A single DNA sample for each individual animal was recovered by pooling equal quantities of the separate DNA preparations and stored at -80°C prior to sequencing.

Purified DNA was used for PCR amplification and sequencing of 16S rRNA genes on an Illumina MiSeq instrument with 2 x 300 base-pair paired-end reads at GATC Biotech (Germany). Universal primers of 16S rRNA genes were used to amplify the hypervariable regions, V3-V5 (V3F (357F), V5R (926R)). In a second PCR, Illumina TruSeq adapters and tag sequences were attached prior to sequencing. Reads have been submitted to the SRA with Accession Number SRP152214.

Bioinformatics and statistical analysis

Sequences were provided in a demultiplexed format and processed using Quantitative Insights Into Microbial Ecology (QIIME) v 1.8.0 [4]. Paired reads were merged (minimum overlap 18, tolerance 5) and quality filtered with default settings. Filtered sequences were clustered into Operational Taxonomic Units (OTUs) at 97% sequence similarity and Chimera sequences removed using USEARCH [14]. The most abundant sequences for each OTU were used as a representative to identify taxonomy by alignment with the GreenGenes database (gg_13_5, PyNAST default) [4, 15]

prior to filtering with default settings. Alpha- and beta-diversity were calculated in QIIME using the OTU table.

Predicted molecular functions were generated from the taxonomy frequencies using PICRUSt [5] following the workflow described at https://github.com/LangilleLab/microbiome_helper/wiki/PICRUSt-workflow. It is important to note that this approach produces only predictions of metabolic function. The OTU table produced by QIIME was converted to .biom format (http://biom-format.org/documentation/biom conversion.html) before a filtering step to remove those entries which do not have an identified organism. The filter-command produced a closed reference .biom table. Entries to this table were normalised to 16S rRNA gene copy number to provide abundance numbers for each OTU. Kyoto Encyclopedia of Genes and Genomes (KEGG) orthologs (KOs) were then predicted prior to them being collapsed at level three to provide Pathway predictions. The abundance data was normalized to the geometric mean [16] of values for 'house-keeping' functions in genetic information processing [5]. To evaluate the significance of particular taxa to defined Pathways (L3) within each group, the predicted contribution of taxa, identified by regression analysis as being connected to the pathway, were summed.

Relative abundance is presented as mean +/- SEM and differences within and between groups were assessed using GraphPad Prism 6 by: Dunn's multiple comparison tests after one-way ANOVA; Bonferroni multiple comparison tests after two-way ANOVA for differences among more than two groups; Dunnett's multiple comparison tests after two-way ANOVA to compare differences between the control group and other groups. A p value of < 0.05 was considered significant. Principal Coordinates Analysis (PCoA), heat map and hierarchical clustering analysis were conducted with the R software package version 3.2.1 (https://cran.r-project.org/bin/windows/base/old/3.2.1) to compare communities of two or more groups.

RESULTS

Induction of obesity and T2D in rats

Rats fed a high-fat diet and injected with vehicle exhibited a significant increase in body weight ($p < 0.05$ vs (versus) all other groups; Fig. 1a). Treatment with a high-fat diet and a low dose of STZ (T2D) induced a significant increase in blood glucose; significant hyperglycaemia was observed 1 week post-STZ injection in T2D rats ($p < 0.05$ vs all other groups) and this was maintained until the end of the study (Fig. 1b). The insulin tolerance test revealed that T2D rats were insensitive to insulin compared to control rats and displayed significant hyperglycaemia for the duration of the test (all $p < 0.05$ vs Controls; Fig. 1c).

Phylogenetic composition and the relative abundance of taxa of the microbiome communities

Basic statistics for the number of reads and clusters of similar sequences for all four groups are shown in Table S2. Determination of the 16S rRNA sequences allowed phylogenetic classification via QIIME of OTUs of the gut microbiota from the level of phylum to family or genus. At phylum level (Fig. S1) *Bacteroidetes* predominate over *Firmicutes* in the control group while similar levels for each were found in the STZ-alone animals. In contrast the proportion of each switch in the Obese and T2D groups and *Firmicutes* now predominate followed by *Bacteroidetes*. The proportion of *Firmicutes* was significantly higher ($p < 0.0001$ and $p < 0.001$) in T2D compared to both control and STZ-alone, while the abundance of *Bacteroidetes* was significantly lower ($p< 0.0001$ and 0.001). *Firmicutes* was enriched ($p < 0.0001$) and *Bacteroidetes* lower ($p < 0.01$) in Obese compared to STZ-alone while it was higher ($p < 0.05$) in STZ-alone vs control (Fig. S1) and ($p < 0.01$) in T2D vs Obese.

At family level *S24-7* family was the most abundant in both control and STZ-alone followed by various other families while in Obese, *Ruminococcaceae* was the predominant family and in T2D, *Lachnospiraceae* was the predominant family (Fig. S2). When comparing bacteria at family levels, no significant differences were found between control and STZ-alone but differences were found for all other pairwise comparisons of the groups (Fig. S3). For instance *Bacteroidaceae* and *Lachnospiraceae* were significantly enriched ($p < 0.0001$) in the T2D vs control and vs STZ-alone, while the abundance of *S24-7* decreased in both Obese and T2D vs control and STZ-alone ($p <$

0.0001). There were significant differences between nine families when Obese was compared to control and with STZ-alone while there were eight significant differences between Obese and T2D.

The abundant bacteria at genus level differ between the four experimental groups and are shown in Fig. 2 and Table S3. In the control animals, the most abundant genera were *Prevotella* from *Prevotellaceae* family, while in the STZ-alone animals, the most abundant genera *Prevotella* and noticeably *Allobaculum* from *Erysipelotrichaceae* family. In the Obese group, *Bacteroides* from *Bacteroidaceae* family, [*Prevotella*] from [*Paraprevotellaceae*] family, *Oscillospira* and *Ruminococcus* from *Ruminococcaceae* family and *Prevotella* occur at highest levels. In T2D, the most abundant genera were *Bacteroides, Prevotella* and *Blautia* from *Lachnospiraceae* family. Fig. 3 shows the comparison of bacterial genera between the groups. Comparison of genera in control vs STZ-alone showed no statistical difference but differences were apparent for all other groups. For instance a higher proportion of *Blautia* and *Bacteroides* were found in T2D vs both control and STZ-alone while *Allobaculum* was higher in T2D vs control ($p < 0.0001$) (Fig. 3). Noticeably the ratio of *Bacteroides/Prevotella* was much higher in Obese and T2D than both control and STZ-alone (Fig. S4).

Differences between genera in bacterial communities or groups were also apparent when data was analysed by PCoA and hierarchical clustering (not shown). The PCoA was conducted in a pairwise manner and is shown in Fig. 4 and reveals spatial separations between the groups. The exception was between control and STZ-alone which could not be resolved and were overlapping or very close to each other (Fig. 4a). In contrast, Obese and T2D rats showed distinct differences when compared to the control group (Fig. 4b and c).

The differences in diversity of taxa across the groups were also shown by measures of both α- and β-diversity. Fig. 5 illustrates α-diversity determined as PD-whole-tree, chao1 and observed-species. Each of the measures showed a significant reduction in diversity in T2D compared to control. α-diversity in T2D was also significantly lower than in Obese rats. The distance-difference of Unweighted and Weighted UniFrac β-diversity was measured for the bacterial community in the individual animals, pairwise, with animals within the same group and between groups (Table S4). Fig. S5 shows the separations of the distance matrix among the four groups with the exception of control vs STZ-alone and Obese vs T2D of the weighted UniFrac distances by PCoA analysis.

The functional microbiome

The potential for bacterial metabolism in each bacterial group has been provided by PICRUSt. The normalised data was analysed by KEGG Category (level 2 e.g. Carbohydrate metabolism) and Pathway (level 3 e.g. Butanoate metabolism), PCoA, heatmaps and hierarchical clustering and results are shown in Fig. 6 and Fig. S6. So as to focus on major functional activities, analysis of these data was made at level 2 and significant differences were found between the four groups in transcription, translation, amino acid metabolism, biosynthesis of other secondary metabolites, carbohydrate metabolism, energy metabolism, enzyme families, glycan biosynthesis and metabolism, metabolism of cofactors and vitamins, nucleotide metabolism and xenobiotic biodegradation and metabolism (Fig. S6). PCoA showed spatial separation of all groups (Fig. 6a). Fig. S6 shows the hierarchical clustering and heatmaps among the four experimental groups and these data show again a clear separation. Because of the role of short-chain fatty acids in gut health, butyrate and propionate metabolism were analysed at level 3. Fig. 6b shows that butyrate production was significantly lower in T2D compared to other groups. The level of propionate was also determined by group and metabolism was significantly reduced in T2D vs both control and Obese (Fig. 6b). Glycolysis/gluconeogenesis, metabolism of starch and sucrose and fructose and mannose and ABC transporters were significantly higher in Obese and T2D vs both control and STZ-alone groups and also T2D vs Obese (Fig. 6c). Processes producing bacterial-derived inflammatory molecules were also affected as shown in Fig. 6d. Bacterial biosynthesis of Lipopolysaccharide (LPS) and LPS biosynthesis proteins were higher in Obese and T2D, while peptidoglycan biosynthesis and bacterial toxins were higher in T2D compared to the other three groups.

Correlation coefficients have been calculated to analyse the relationship of SCFA metabolism and LPS biosynthesis to individual gut microbiota (Fig. S7). It was found that *Turicibacter* genus and undefined genus of both *S24-7* and *Peptostreptococcaceae* families were positively correlated to the butyrate production while genus of *Blautia,* and *[Ruminococcus]* and unclassified genus of *Lachnospiraceae* family were negatively associated with butyrate production. Both *Ruminococcus* and [*Prevotella*] (without STZ-alone) were positively linked to propionate metabolism. Also, *Bacteroides* was positively correlated with LPS biosynthesis. The relative abundances of bacteria that either produce butyric acid or propionic acid, are shown in Fig. S7.

DISCUSSION

This study describes altered composition and metabolic potential of gut microbiota in rats fed with a diet containing high fat content that induced obesity or in combination with a single, low-dose STZ injection that induced T2D. Rats fed with HFD plus low-dose STZ developed insulin-insensitivity and hyperglycaemia consistent with the phenotype of T2D while STZ-alone caused no lasting physiological changes. Diabetic rats had weight loss as a result of loss of calories from sugar in the urine and the consequence of fat cell breakdown for energy production [9]. This is in contrast to Obese rats who exhibited a significant increase in body weight but with no change in blood glucose levels.

In this study the most abundant bacterial phyla were the *Firmicutes* and the *Bacteroidetes* (Fig. S1) and there was a significant change in the relative abundance of these phyla in the diabetic animals. Similar changes have been observed in obesity in human [17].

PICRUSt metabolic analysis indicates potential bacterial-derived metabolic capabilities and specifically on metabolism of SCFA, including butyrate or propionate and on inflammatory molecules that are increased in both the obese and diabetic condition. In this study predictions for levels of butyrate indicate that this metabolite would be decreased in both the Obese and T2D rats compared to those on the normal diet. At variance with this are the results from a meta-analysis of 8 data sets using PICRUSt to make predictions and found that these pathways would be increased [18]. It is likely that the conflict between our predictions and those of Jiao et al [18] are a consequence of their use of a mixture of genera (5 mice, 3 rats) with variant species of each genus. It is generally accepted that butyrate production is beneficial and thus decreased production in Obese and T2D may be expected and those predictions could be tested in further experimentation. Butyrate is protective of the single cell-layer of epithelial cells along with its mucin coating while this barrier is compromised by inflammatory molecules [19]. In this study, the most abundant taxa in the control animals were unclassified genus of *S24-7* family, *Turicibacter* genus and unclassified genus of *Peptostreptococcaceae* family (Fig. 2). PICRUSt positively linked these bacteria to butyrate production (Fig. S7) and they have been identified as butyrate-producing bacteria (*S24-7* family [20], *Peptostreptococcaceae* family [21], and *Turicibacter* genus [22]). Butyrate is produced as a bacterial metabolite and contributes to the integrity and thickness of the whole mucosal barrier [23] since it

promotes the synthesis and secretion of mucin into the intestine [9, 24], stimulates Claudin-1, a protein of tight-junctions [25] and acts as an anti-inflammatory [26]. Butyrate is also important in the activation of host GRP41/43 that elicits production of appetite-suppressing PYY and insulin-producing GLP1 [27]. In the STZ-alone rats, there was no significant difference in bacterial profile compared to control at the level of genus with the exception of an increase of *Allobaculum* genus (Fig. 2 and Fig. 3). A study of gut microbiota in mice [28] found that mice fed with low fat diet showed an enrichment of this genus. In fact, *Allobaculum* in the intestine encourages mucin release because this bacterium produces butyrate [29]. We found no difference in our predictions between Control and Obese groups for propionate metabolism again in contrast to the predictions of Jiao et al [18] but consistent with the predictions of Lee and Ko [30] who found an improvement in metabolic parameters and increased propionate in metformin administered mice on a high fat diet. Our results for predictions on butyrate and propionate are also in accord with a chemical study of human subjects and both of these short chain fatty acids were decreased in faecal material from patients with T2D [31]. Additionally, a study employing genome-wide genotyping and gut metagenomic sequencing of a large panel of human subjects found a positive association between butyrate production and good insulin response to oral glucose administration [32].

The data from Obese rats highlighted the significant increase in *Firmicutes* phylum and associated decrease in *Bacteroidetes*. There was a significant enrichment of genus *Bacteroides* and a reduction of genus *Prevotella* in Obese rats compared to both control and STZ-alone groups. *Bacteroides* is a Gram-negative bacterium and is able to digest a variety of polysaccharides [33] producing fructose from fructans and then saccharolytic fermentation, produces acetate which is used for methanogenesis by *Methanobrevibacter smithii* [34]. Acetate is also utilised in energy metabolism by the host leading to increased adipose tissue. In the Obese rats, there was enrichment of [*Prevotella*]/ [*Paraprevotellaceae*] and this bacterium was predicted to be positively associated with propionate metabolism (Fig. S7). *Ruminococcus* and *Oscillibacter* were increased in the Obese rats (Fig. 3). Some *Ruminococcus* sp are acetate producers [35] and *R. bromii* and *R. obeum* were associated with obesity [36] and here a positive correlation between *Ruminococcus* and predicted propionate metabolism was found (Fig. S7). There are three pathways of propionate metabolism and the association between *Ruminococcus* sp. and one of these has recently been confirmed in the human gut microbiota [37]. Reichardt and his colleagues found that the propanediol pathway occurs in some

283 species of *Ruminococcus* genus and *Lachnospiraceae* family. Krych et al., [38] showed that the
284 occurrence of bacteria such as *Lachnospiraceae* family, and both genera *Oscillospira* and
285 *Ruminococcus* from *Ruminococcaceae* family are associated with the promotion of diabetes.

286 Perhaps the most dramatic changes in bacterial communities were found in the rats in which T2D had
287 been induced. Further these changes appear to be associated with the physiological state expected of a
288 diabetic animal. The diabetic rats had a decreased ratio of *Firmicutes/ Bacteroidetes* despite an
289 increase of *Bacteroides*. The increase of *Bacteroides* was positively correlated with predicted LPS
290 biosynthesis. *Blautia* was also increased and is a gram-positive, non-sporulating coccobacillus
291 belonging to the *Firmicutes* phylum [39]. In humans *Blautia* was the predominant genus in pre-
292 diabetic and T2D patients [40] and plays a vital role in the metabolism of glucose which it converts to
293 acetate, lactate, hydrogen, ethanol and succinate in the gut [39]. A recent report by Ozato et al., [41]
294 found that visceral fat in individuals in a Japanese population was inversely associated with *Blautia*.
295 In our study *Blautia* was not significantly different in the Obese animals compared to the Control,
296 however, our measure of Obesity was body weight rather than visceral fat. The predicted increase of
297 bacterial gut-derived inflammatory molecules (for instance, LPS, flagellin and peptidoglycans) and
298 predicted decreased butyrate production would be likely to be associated with the causation of
299 inflammation and T2D [42]. Increased permeability of the gut membrane and low level inflammation
300 caused by LPS and bacterial toxins has been reviewed [43].

301 As a working hypothesis we propose that the relationship between diet and the role of either beneficial
302 or harmful gut microbiota in Wistar rats is that summarized in Fig 7. The taxonomy of the bacterial
303 communities and the bacterial metabolic capabilities were comparable in both control groups and
304 predicted to promote the production of mucin and protection of the gut barrier layer. On the other
305 hand, there were significant differences in the bacterial communities and metabolic potential in the
306 Obese and T2D rats. Changes from a high ratio of *Prevotella/ Bacteroides* in controls to a low ratio in
307 the T2D animals (data not shown) are associated with a healthy to diabetic transition and similar
308 conclusions have been reached in humans and mice [44]. This is diet associated and these differences
309 were observed when comparing both groups on a normal diet to both groups on a high fat diet. In
310 humans *Prevotella* is associated with plant-based diets [45] and the normal diet provided for control
311 rats is derived from a plant-based source including soya, wheat and barley

(http://www.sdsdiets.com/pdfs/RM1P-E-FG.pdf). However, single-component diet change does not itself bring about a change in *Prevotella/ Bacteroides* ratio or a loss of body weight [46]. Intervention is clearly context specific and the development of therapies for metabolic diseases will need to be mindful of the antagonistic interaction between *Prevotella and Bacteroides* [47], dietary presence of complex carbohydrates or presence of a fat- and protein-rich diet.

Obesity predisposes to T2D but this is not the case in all instances of the disease [48, 49]. Identifying the pathway from obesity to T2D and the role of the gut microbiome is especially difficult because of the interaction of a large number of gut organisms, many of which have not been identified, with each other and the host and the balance between harmful and beneficial interactions [50]. Seeking links between obesity and T2D is not revealed by this study but some clues are provided. *Blautia* is present in Obese rats and significantly elevated in T2D rats. This is predicted to disrupt carbohydrate and glucose metabolism that reduces butyrate production with an increase of other SCFA and these products may contribute to energy capture by the host [51, 52]. The one common feature of the microbial changes in both Obese and T2D rats is the increase of *Bacteroides*. *Bacteroides* are uniquely able to regulate the expression of genes for polysaccharide degradation and uptake and these are determined by the identity and availability of specific polysaccharides. They do this through different gene cassettes that are differentially controlled by intermediates of the breakdown process and may differ between species [53]. Thus we hypothesise that species within *Blautia* and *Bacteroides* are significantly associated with both Ob and T2D possibly as a mechanistic driver. The animal models share many characteristics that have been described from studies of humans either of obesity or T2D and future work may profit from a focus on *Blautia* and *Bacteroides* in these animal models and identifying which specific species in the two genera are altered.

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Conflict of Interest:

KSI declares that he has no conflict of interest.

NB declares that she has no conflict of interest.

SD declares that she has no conflict of interest.

SL declares that she has no conflict of interest.

JS declares that she has no conflict of interest.

JAC declares that he has no conflict of interest.

Compliance with Ethical Standards and Ethical approval

All applicable international, national, and/or institutional guidelines for the care and use of animals were followed. Rats were treated with full approval of the Institute's Animal Ethic's and Welfare Committee; the procedures complied with UK Animal Scientific Procedures Act (1986) and were approved by the Home Office.

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506

507

Figure Legends

Fig. 1 Total weight gain (g) from week 0 to week 12 (a), blood glucose (mmol/L) measured on week 12 (b) in Control, STZ-alone, Obese and Diabetic rats (n=6/group), and the insulin tolerance test compared Diabetic vs Control rats (c). Data are mean ± SEM. Significant difference from all other groups

Fig. 2 Bacterial taxonomy and abundance of the gut metagenome (a) at genus level between the four experimental groups

Fig. 3 Differences in abundance of genera between the four experimental groups

Fig. 4 Comparison of bacterial communities at genus level in the four experimental groups based on PCoA

Comparisons are pairwise for individual rats in each group (n=6); Control green, STZ-alone red, Obese blue and Diabetic black.

Fig. 5 Bacterial α-diversity of bacterial communities in the four experimental groups

Dunn's multiple comparisons test was used to determine the relationship of α-diversity between the microbiome of the rat groups (n=6).*$p<0.05$, **$p<0.01$, and ***$p<0.001$.

Fig. 6 PICRUSt analysis for predictions of the functional microbiome of each group

PICRUSt was conducted at level 3. PCoA was used with individuals (a). The Mann Whitney test was used to estimate the significant differences; Metabolism of SCFA (butyrate and propionate) (b); energy related metabolism (glycolysis/ gluconeogenesis, starch and sucrose metabolism, fructose and mannose metabolism, and ABC transporters) (c) and processes producing bacterial-derived inflammatory molecules (d) Bacterial biosynthesis of lipopolysaccharide (LPS), LPS biosynthesis proteins, Bacterial toxins and Peptidoglycan biosynthesis.

Fig. 7 A model of the interactions between gut and microbial communities in normal (a), diabetic (b) and obese (c) conditions

Supplementary Figure Legends

Figure S1: Bacterial taxonomy at phylum level (a) and differences in abundance of phyla (b)

The Bonferroni's comparison test was used to estimate the relationship of bacterial phyla between the groups (n=6/ group).

Figure S2: Bacterial taxonomy at family level

Figure S3 Differences in abundance of family between the four experimental groups

The Bonferroni's comparison test was used to estimate the relationship of bacterial families between the groups (n=6/ group).

Figure S4 Ratio of Bacteroides/Prevotella among the four groups

Figure S5: Bacterial β-diversity (weighted UniFrac) of the microbial communities

The bacterial communities of individual rats based on the weighted UniFrac by PCoA.

Figure S6: PICRUSt analysis for predictions of microbial communities at level 3; Hierarchical clustering (a) and heatmap (b)

Hierarchical clustering and Heat map analysis by R statistic (n=6). The Hierarchical clustering was used to confirm the similarity of bacterial function between the groups. Heatmap was use to illustrate the relative abundant of each individual bacterial function among these four groups.

Figure S7: Correlations between bacteria and predicted metabolic activities

Linear correlation Pearson coefficients and nonparametric Spearman were used to show the relationship of gut bacteria either with SCFAs (butyric acid (a), propionic acid (b)) and LPS biosynthesis (c) and the relative abundance of bacteria that produce butyric acid (d) and propionic acid (e).

555 Supplementary Table Legends

556 Table S1: Composition (% of total) of the standard animal house diet (RM1) and high fat diet (HFD):
557 (a) Nutrients (b) carbohydrate, fiber and non-starch polysaccharides and (c) fatty acids

558 Table S2: Quality control and OTU assignments of the NGS Illumina MiSeq reads of the four rat
559 experimental groups

560 The data shows numbers of reads for individual animals after quality trimming (phred > 20), merging
561 of pairs, similarity-clustering and chimera identification. OTUs were assigned before filtering to show
562 best hit only. Key: NDV = normal diet; NDS = normal diet plus STZ injection; HFV = high fat diet
563 (Obese); HFS = high fat diet plus STZ injection (Diabetic).

564 Table S3: Bacterial taxonomy at genera level
565 The Phylogenetic analysis at genus level of faecal 16S rRNA for the four experimental groups (n=6/
566 group). The mean abundance (%) from the bacterial taxonomic profiling is shown. Values <0.01%
567 show as 0.00 due to decimal point truncation.

568 Key Control= normal diet; STZ-alone= normal diet plus STZ injection; Obese= high fat diet and
569 Diabetic= high fat diet plus STZ injection.

570

571 Table S4: Differences of bacterial beta diversity: unweighted (a) and weighted (b) in the individual
572 animal samples

573 Key: NDV = normal diet; NDS = normal diet plus STZ injection; HFV = high fat diet (Obese); HFS =
574 high fat diet plus STZ injection (Diabetic).

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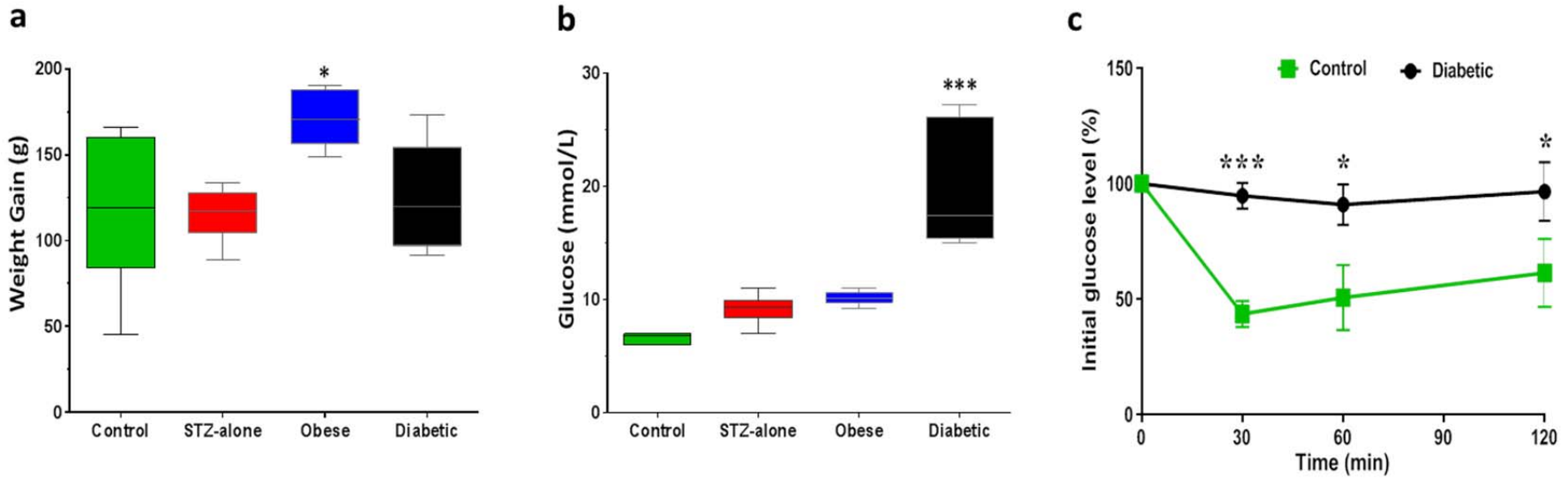


* P < 0.05; *** P < 0.001

Fig. 1

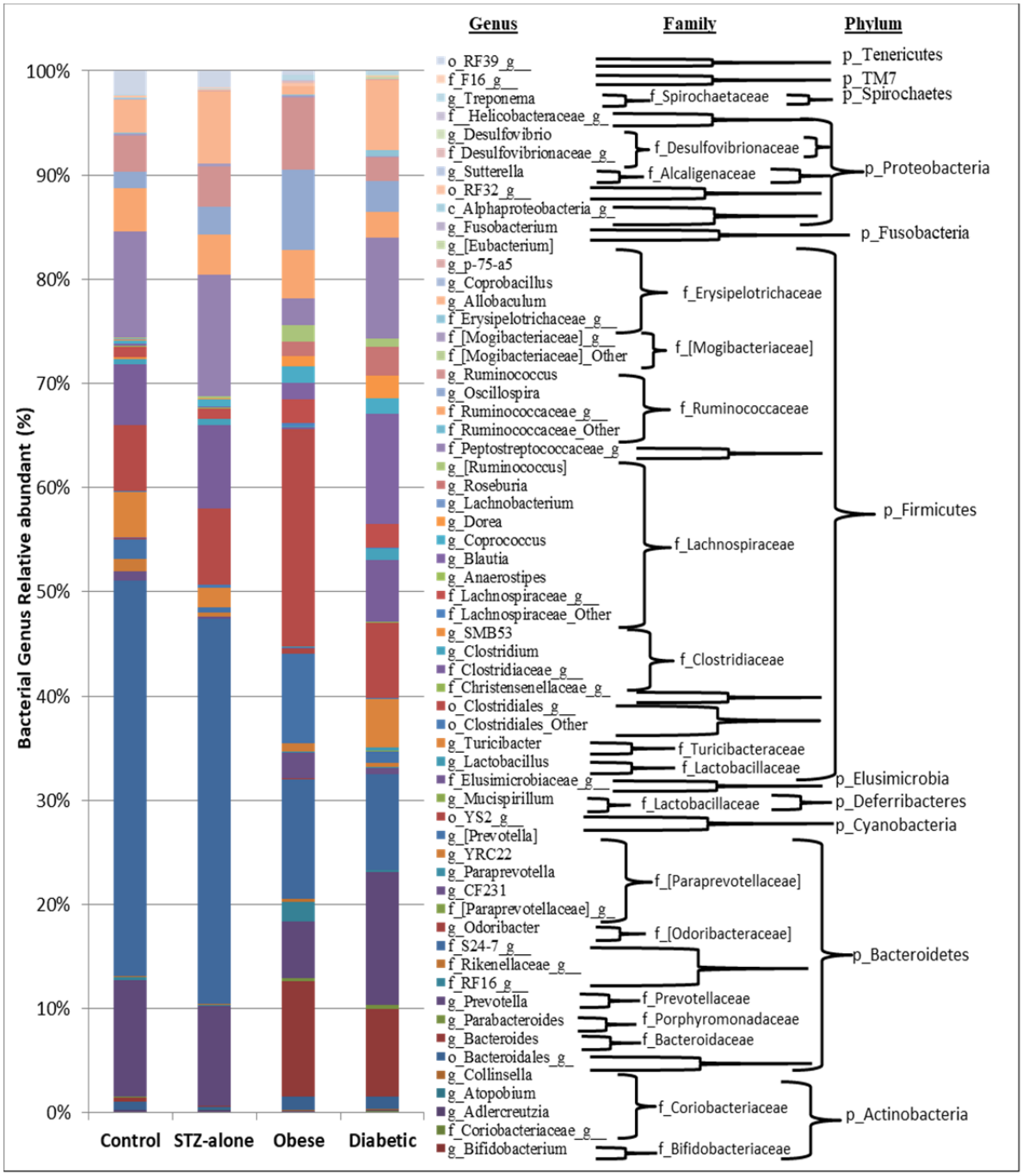
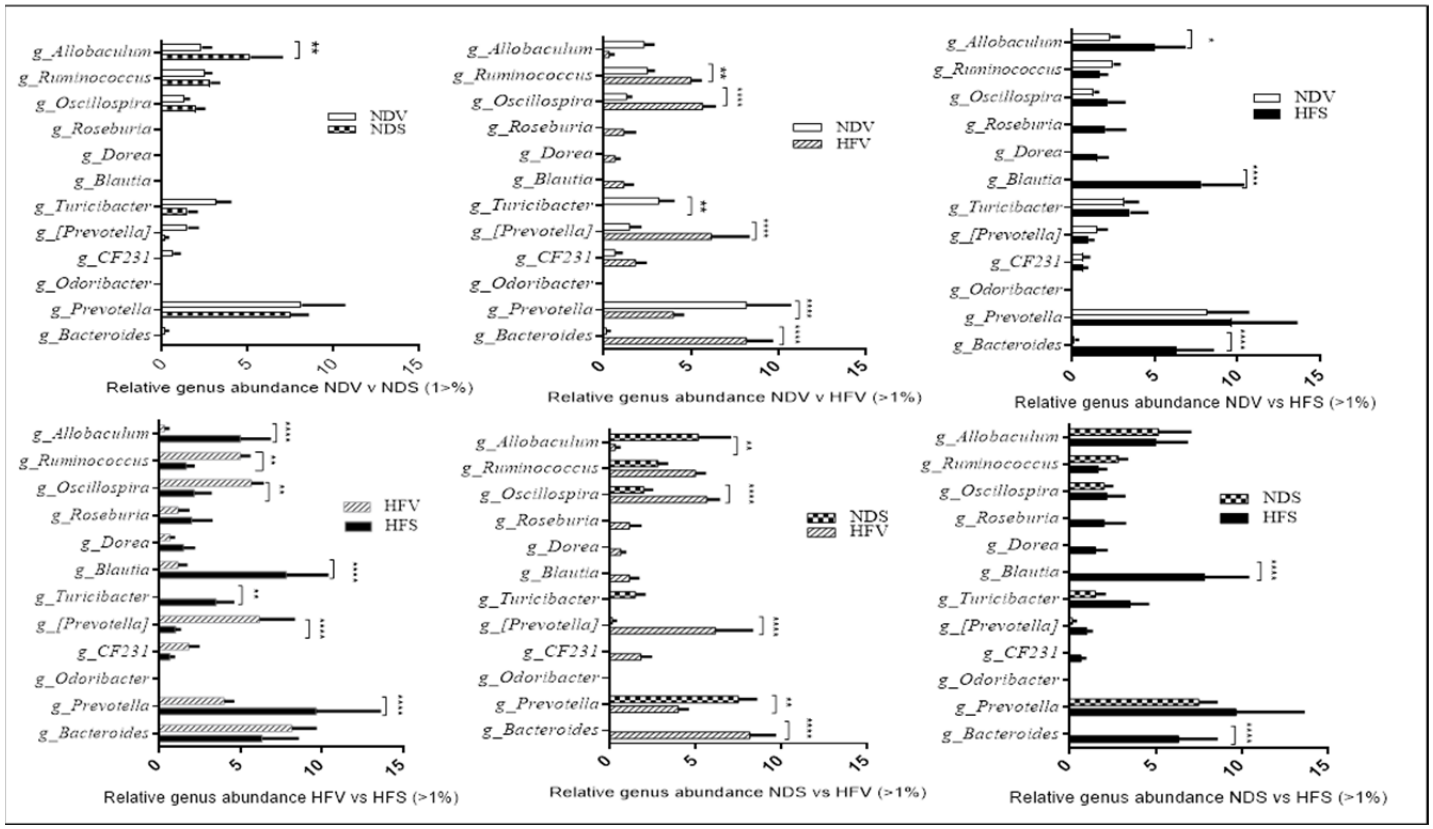

Genus
Family
Phylum
Bacterial Genus Relative abundant (%)
100%
90%
80%
70%
60%
50%
40%
30%
20%
10%
0%
Control
STZ-alone
Obese
Diabetic
o_RF39_g__
f_F16_g__
g_Treponema
f__Helicobacteraceae_g_
g_Desulfovibrio
f_Desulfovibrionaceae_g_
g_Sutterella
o_RF32_g__
c_Alphaproteobacteria_g_
g_Fusobacterium
g_[Eubacterium]
g_p-75-a5
g_Coprobacillus
g_Allobaculum
f_Erysipelotrichaceae_g__
f_[Mogibacteriaceae]_g__
f_[Mogibacteriaceae]_Other
g_Ruminococcus
g_Oscillospira
f_Ruminococcaceae_g__
f_Ruminococcaceae_Other
f_Peptostreptococcaceae_g
g_[Ruminococcus]
g_Roseburia
g_Lachnobacterium
g_Dorea
g_Coprococcus
g_Blautia
g_Anaerostipes
f_Lachnospiraceae_g__
f_Lachnospiraceae_Other
g_SMB53
g_Clostridium
f_Clostridiaceae_g__
f_Christensenellaceae_g_
o_Clostridiales_g__
o_Clostridiales_Other
g_Turicibacter
g_Lactobacillus
f_Elusimicrobiaceae_g__
g_Mucispirillum
o_YS2_g__
g_[Prevotella]
g_YRC22
g_Paraprevotella
g_CF231
f_[Paraprevotellaceae]_g_
g_Odoribacter
f_S24-7_g__
f_Rikenellaceae_g__
f_RF16_g__
g_Prevotella
g_Parabacteroides
g_Bacteroides
o_Bacteroidales_g_
g_Collinsella
g_Atopobium
g_Adlercreutzia
f_Coriobacteriaceae_g__
g_Bifidobacterium
f_Spirochaetaceae
f_Desulfovibrionaceae
f_Alcaligenaceae
f_Erysipelotrichaceae
f_[Mogibacteriaceae]
f_Ruminococcaceae
f_Lachnospiraceae
f_Clostridiaceae
f_Turicibacteraceae
f_Lactobacillaceae
f_Lactobacillaceae
f_[Paraprevotellaceae]
f_[Odoribacteraceae]
f_Prevotellaceae
f_Porphyromonadaceae
f_Bacteroidaceae
f_Coriobacteriaceae
f_Bifidobacteriaceae
p_Tenericutes
p_TM7
p_Spirochaetes
p_Proteobacteria
p_Fusobacteria
p_Firmicutes
p_Elusimicrobia
p_Deferribacteres
p_Cyanobacteria
p_Bacteroidetes
p_Actinobacteria

Fig. 2



*p<0.05, ** p<0.01, ***p<0.001, ****p< 0.0001

Fig. 3

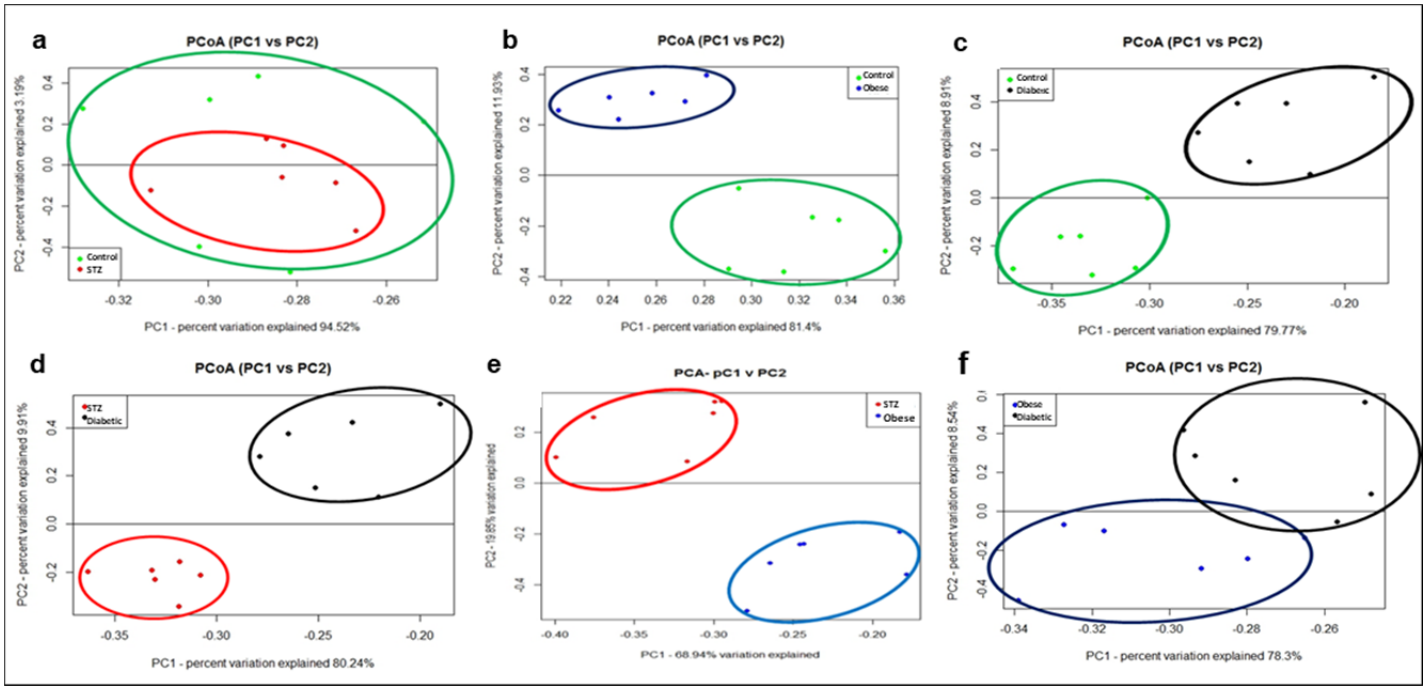
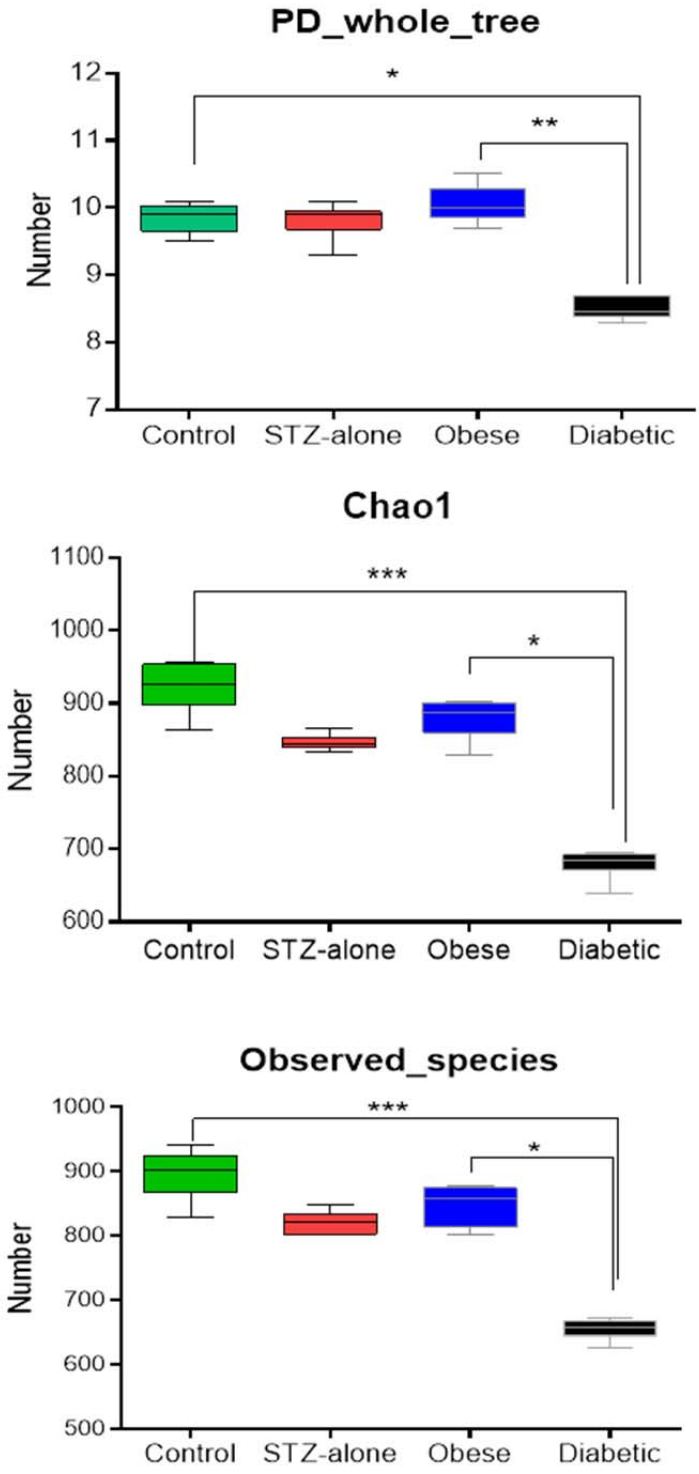
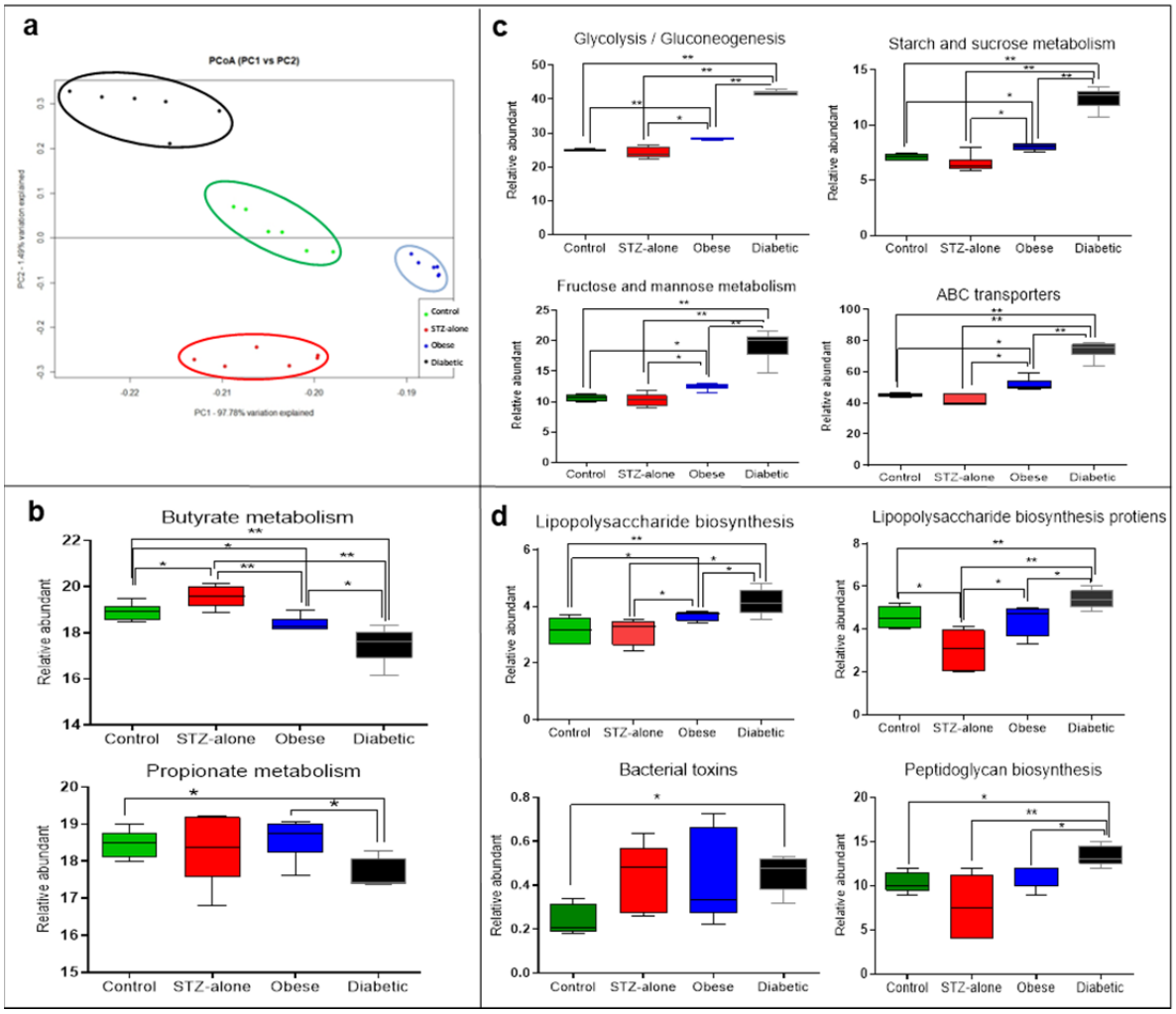

a
PCoA (PC1 vs PC2)
PC2 - percent variation explained 3.19%
PC1 - percent variation explained 94.52%
Control
STZ
b
PCoA (PC1 vs PC2)
PC2 - percent variation explained 11.93%
PC1 - percent variation explained 81.4%
Control
Obese
c
PCoA (PC1 vs PC2)
PC2 - percent variation explained 8.91%
PC1 - percent variation explained 79.77%
Control
Diabetic
d
PCoA (PC1 vs PC2)
PC2 - percent variation explained 9.91%
PC1 - percent variation explained 80.24%
STZ
Diabetic
e
PCA- pC1 v PC2
PC1 - 19.85% variation explained
PC1 - 68.94% variation explained
STZ
Obese
f
PCoA (PC1 vs PC2)
PC2 - percent variation explained 8.54%
PC1 - percent variation explained 78.3%
Obese
Diabetic

Fig. 4



*p<0.05, **p<0.01, and ***p<0.001

Fig. 5



*P<0.05, ** P<0.01

Fig. 6

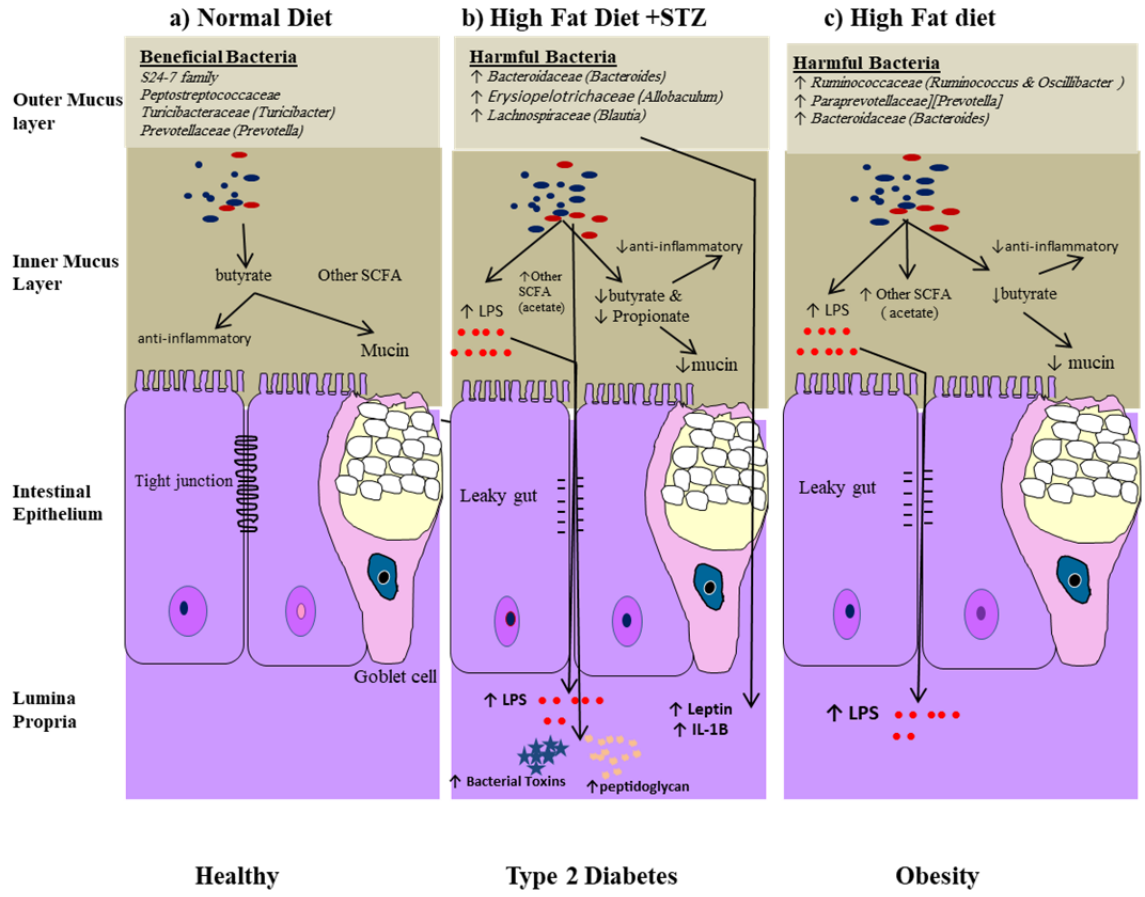

a) Normal Diet
b) High Fat Diet +STZ
c) High Fat diet
Outer Mucus layer
Inner Mucus Layer
Intestinal Epithelium
Lumina Propria
Beneficial Bacteria
S24-7 family
Peptostreptococcaceae
Turicibacteraceae (Turicibacter)
Prevotellaceae (Prevotella)
butyrate
Other SCFA
anti-inflammatory
Mucin
Tight junction
Goblet cell
Harmful Bacteria
↑ Bacteroidaceae (Bacteroides)
↑ Erysiopelotrichaceae (Allobaculum)
↑ Lachnospiraceae (Blautia)
↓anti-inflammatory
↑Other SCFA (acetate)
↑ LPS
↓butyrate & ↓ Propionate
↓mucin
Leaky gut
↑ LPS
↑ Leptin
↑ IL-1B
↑ Bacterial Toxins
↑peptidoglycan
Harmful Bacteria
↑ Ruminococcaceae (Ruminococcus & Oscillibacter)
↑ Paraprevotellaceae][Prevotella]
↑ Bacteroidaceae (Bacteroides)
↓anti-inflammatory
↑ LPS
↑ Other SCFA (acetate)
↓butyrate
↓ mucin
Leaky gut
↑ LPS
Healthy
Type 2 Diabetes
Obesity

Fig. 7

Supplementary material for:

Characterisation of gut microbiota of obesity and type 2 diabetes in rodent model

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Table and figures of contents

Table S1: Composition (% of total) of the standard animal house diet (RM1) and high fat diet (HFD): (a) Nutrients (b) carbohydrate, fiber and non-starch polysaccharides and (c) fatty acids

a) Nutrients

	Standard Diet (RM1) (%)	HFD (%)
Moisture	10	7.46
Crude Oil	2.71	22.27
Crude Protein	14.38	19.87
Crude fibre	4.65	3.91
Ash	6	5.24
Nitrogen Free Extract	61.73	41.02

b) Carbohydrate, fibre, and non-starch polysaccharide

	Standard Diet (RM1) (%)	HFD (%)
Pectin	1.52	0.46
Hemicellulose	10.17	3.17
Cellulose	4.32	4.85
Lignin	1.68	0.43
Starch	44.97	34.74

c) Fatty Acids

	RM1 (%)	Total (%)	HFD (%)	Total (%)
Saturated Fatty Acid				
C12:0 Lauric	0.02	0.51	0.03	7
C14:0 Myristic	0.14		0.37	
C16:0 Palmitic	0.31		4.56	
C18:0 Stearic	0.04		2.04	
Monounsaturated Fatty Acids				
C14:1 Myristoleic	0.02	0.88	0.03	6.83
C16:1 Palmitoleic	0.09		0.11	
C18:1 Oleic	0.77		6.69	
Polyunsaturated Fatty Acids				
C18:2 Linoleic	0.69	0.88	1.95	2.09
C18:3 Linolenic	0.06		0.11	
C20:4 Arachidonic	0.13		0.03	
C22:5 Clupanodonic	0.00		0.00	

Table S2: Quality control and OTU assignments of the NGS Illumina MiSeq reads of the four rat experimental groups

A

NDV	NDV1		NDV2		NDV3		NDV4		NDV5		NDV6		NDV Total	NDV average
Description	Read pairs	% Read pairs	Read pairs	% Read pairs	Read pairs	% Read pairs	Read pairs	% Read pairs	Read pairs	% Read pairs	Read pairs	% Read pairs	Read pairs	% Read pairs
Total	780,941	100	737,400	100	716,068	100	737,118	100	644,102	100	796,571	100	4412200	100
Cleaned	231,294	29.6	229,708	31.2	207,409	29	219,892	29.8	185,833	28.9	232,308	29.2	1306444	29.62
Cleaned (orphan)	309,687	39.7	307,886	41.8	293,119	40.9	300,765	40.8	263,972	41	331,626	41.6	1807055	40.92
Merged by overlapping	228,867	29.3	227,768	30.9	205,316	28.7	217,153	29.5	184,050	28.6	229,897	28.9	1293051	29.32
Clustered by similarity	150,689	19.3	191,096	25.9	178,638	24.9	146,366	19.9	115,335	17.9	150,764	18.9	932888	21.13
Chimeric	17,410	2.2	25,966	3.5	17,913	2.5	13,754	1.9	12,903	2	10,382	1.3	98328	2.23
Final high quality	133,279	17.1	165,130	22.4	160,725	22.4	132,612	18	102,432	15.9	140,382	17.6	834560	18.90
OTU assigned	105,777	79.4	140,650	85.2	133,319	82.9	106,433	80.3	80,746	78.8	110,486	78.7	677411	80.88
Filter passed OTUs (best hit only)	22,682	17	34,358	20.8	37,209	23.2	17,652	13.3	10,348	10.1	18,763	13.4	141012	16.3
Filter passed OTUs (all hits)	22,682	17	34,350	20.8	37,201	23.1	17,652	13.3	10,348	10.1	18,763	13.4	140996	16.28

b

NDS	NDS1		NDS3		NDS4		NDS5		NDS6		NDS7		NDS Total	NDS average
Description	Read pairs	% Read pairs	Read pairs	% Read pairs	Read pairs	% Read pairs	Read pairs	% Read pairs	Read pairs	% Read pairs	Read pairs	% Read pairs	Read pairs	% Read pairs
Total	932,125	100	869,327	100	909,084	100	884,318	100	727,318	100	993,913	100	5316085	100
Cleaned	288,646	31	262,287	30.2	270,190	29.7	293,657	33.2	189,592	26.1	290,452	29.2	1594824	29.9
Cleaned (orphan)	373,270	40	358,907	41.3	367,897	40.5	346,172	39.1	313,070	43	397,420	40	2156736	40.65
Merged by overlapping	286,361	30.7	259,753	29.9	267,166	29.4	291,732	33	187,310	25.8	287,512	28.9	1579834	29.62
Clustered by similarity	208,704	22.4	189,540	21.8	216,130	23.8	256,326	29	161,039	22.1	234,675	23.6	1266414	23.78
Chimeric	21,152	2.3	32,822	3.8	28,010	3.1	37,134	4.2	7,430	1	17,777	1.8	144325	2.7
Final high quality	187,552	20.1	156,718	18	188,120	20.7	219,192	24.8	153,609	21.1	216,898	21.8	1122089	21.08
OTU assigned	149,695	79.8	124,893	79.7	157,651	83.8	183,926	83.9	129,112	84.1	182,741	84.3	928018	82.6
Filter passed OTUs (best hit only)	24,261	12.9	31,940	20.4	48,774	25.9	40,427	18.4	33,804	22	33,137	15.3	212343	19.15
Filter passed OTUs (all hits)	24,257	12.9	31,937	20.4	48,774	25.9	40,412	18.4	33,792	22	33,121	15.3	212293	19.15

Continued Table S2

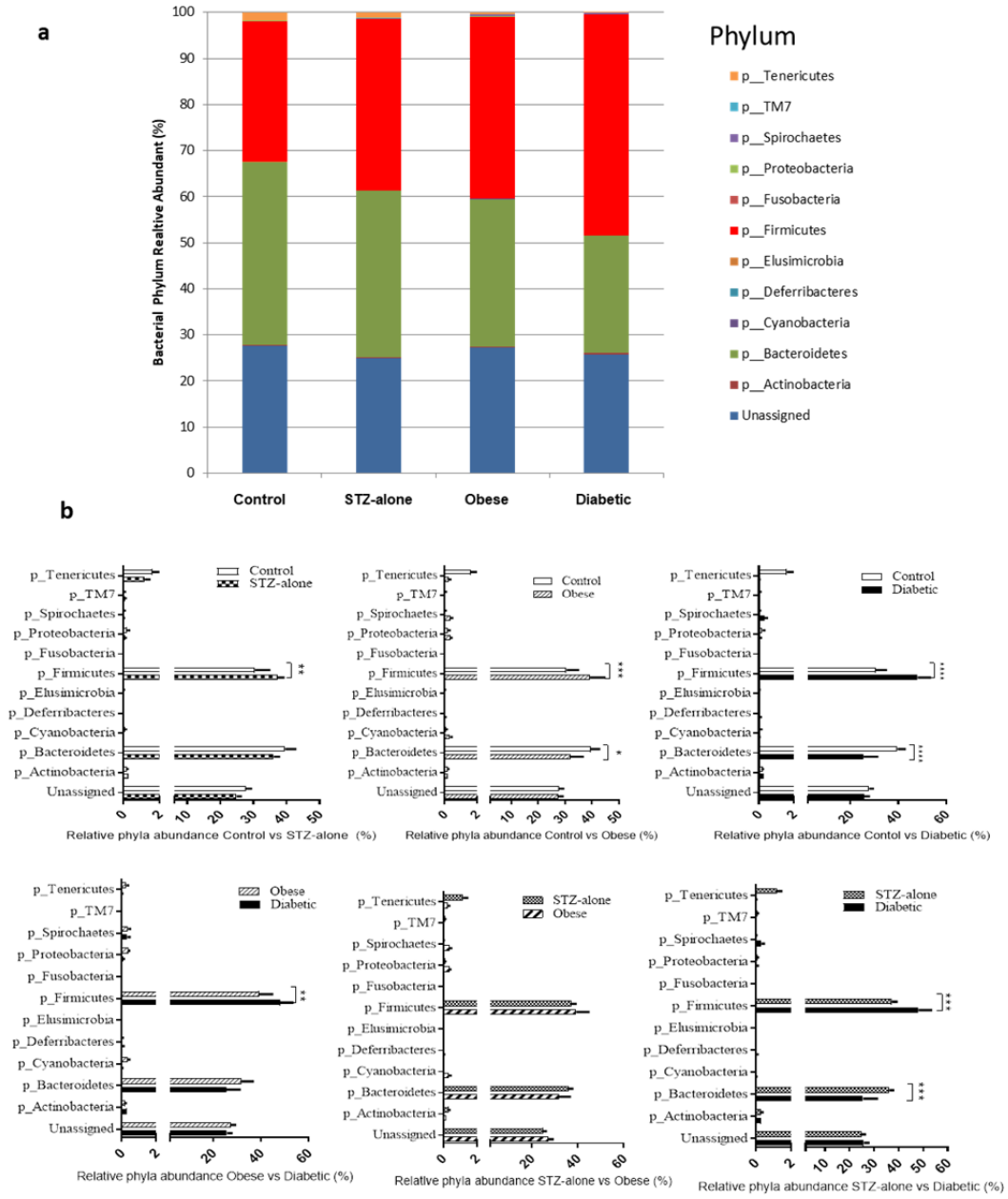
C

HFV	HFV1		HFV2		HFV3		HFV4		HFV5		HFV6		HFV Total	HFV Average
Description	Read pairs	% Read pairs	Read pairs	% Read pairs	Read pairs	% Read pairs	Read pairs	% Read pairs	Read pairs	% Read pairs	Read pairs	% Read pairs	Read pairs	% Read pairs
Total	878,903	100	841,564	100	1,031,154	100	546,750	100	915,912	100	850,833	100	5065116	100
Cleaned	249,017	28.3	243,693	29	278,030	27	163,812	30	255,233	27.9	253,862	29.8	1443647	28.67
Cleaned (orphan)	349,489	39.8	340,241	40.4	416,247	40.4	220,348	40.3	384,532	42	337,435	39.7	2048292	40.43
Merged by overlapping	246,691	28.1	241,673	28.7	275,510	26.7	162,487	29.7	253,048	27.6	251,584	29.6	1430993	28.4
Clustered by similarity	156,214	17.8	192,396	22.9	177,636	17.2	124,932	22.8	167,755	18.3	216,273	25.4	1035206	20.73
Chimeric	15,427	1.8	22,686	2.7	15,126	1.5	20,483	3.7	30,723	3.4	19,366	2.3	123811	2.57
Final high quality	140,787	16	169,710	20.2	162,510	15.8	104,449	19.1	137,032	15	196,907	23.1	911395	18.2
OTU assigned	106,370	75.6	140,862	83	124,745	76.8	86,469	82.8	109,546	79.9	164,443	83.5	732435	80.27
Filter passed OTUs (best hit only)	9,652	6.9	29,884	17.6	15,635	9.6	21,614	20.7	31,066	22.7	38,746	19.7	146597	16.2
Filter passed OTUs (all hits)	9,652	6.9	29,876	17.6	15,635	9.6	21,609	20.7	31,066	22.7	38,732	19.7	146570	16.2

D

HFS	HFS1		HFS2		HFS3		HFS4		HFS5		HFS6		HFS Total	HFS Average
Description	Read pairs	% Read pairs	Read pairs	% Read pairs	Read pairs	% Read pairs	Read pairs	% Read pairs	Read pairs	% Read pairs	Read pairs	% Read pairs	Read pairs	% Read pairs
Total	879,377	100	870,058	100	830,865	100	785,820	100	893,885	100	926,068	100	5186073	100
Cleaned	269,265	30.6	256,001	29.4	254,992	30.7	251,249	32	289,821	32.4	296,895	32.1	1618223	31.2
Cleaned (orphan)	346,312	39.4	336,857	38.7	321,220	38.7	306,594	39	336,654	37.7	348,570	37.6	1996207	38.52
Merged by overlapping	266,270	30.3	253,241	29.1	252,249	30.4	249,861	31.8	287,632	32.2	293,742	31.7	1602995	30.92
Clustered by similarity	230,592	26.2	210,571	24.2	209,732	25.2	183,267	23.3	218,448	24.4	229,017	24.7	1281627	24.67
Chimeric	41,212	4.7	39,929	4.6	51,804	6.2	47,859	6.1	63,757	7.1	67,893	7.3	312454	6
Final high quality	189,380	21.5	170,642	19.6	157,928	19	135,408	17.2	154,691	17.3	161,124	17.4	969173	18.67
OTU assigned	166,818	88.1	144,466	84.7	130,191	82.4	111,649	82.5	120,478	77.9	124,040	77	797642	82.1
Filter passed OTUs (best hit only)	87,557	46.2	66,175	38.8	68,890	43.6	51,956	38.4	54,679	35.3	73,728	45.8	402985	41.35
Filter passed OTUs (all hits)	87,554	46.2	66,173	38.8	68,888	43.6	51,951	38.4	54,679	35.3	73,728	45.8	402973	41.35

The data shows numbers of reads after quality trimming (phred > 20), merging of pairs, similarity-clustering and chimera identification. OTUs were assigned before filtering to show best hit only. Key: NDV = normal diet; NDS = normal diet plus STZ injection; HFV = high fat diet (Obese); HFS = high fat diet plus STZ injection (Diabetic).



*P<0.05, ** P<0.01, ***P<0.001, ****P< 0.0001

Figure S1: Bacterial taxonomy at phylum level (a) and differences in abundance of phyla (b)

The Bonferroni's comparison test was used to estimate the relationship of bacterial phyla between the groups (n=6/ group).

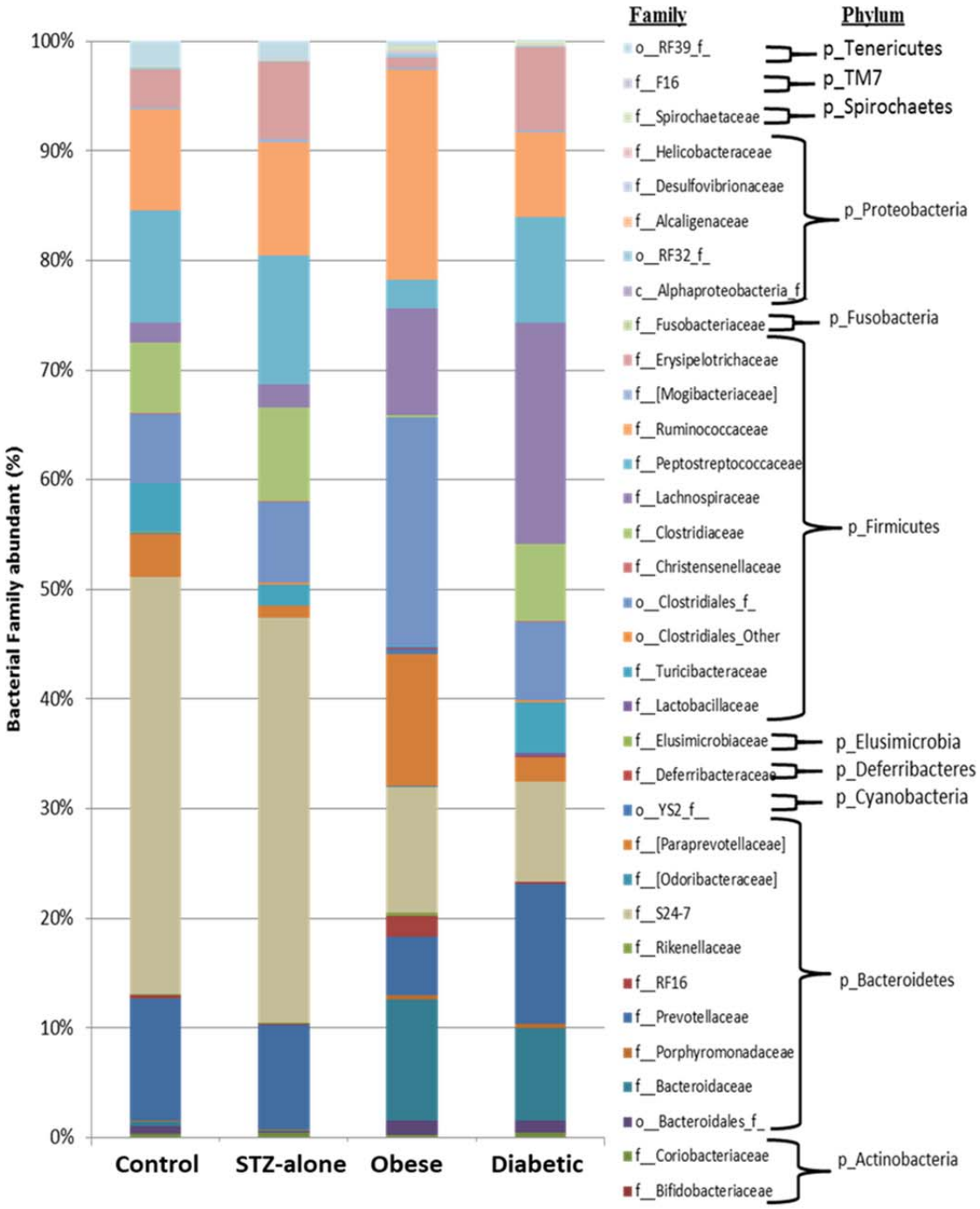
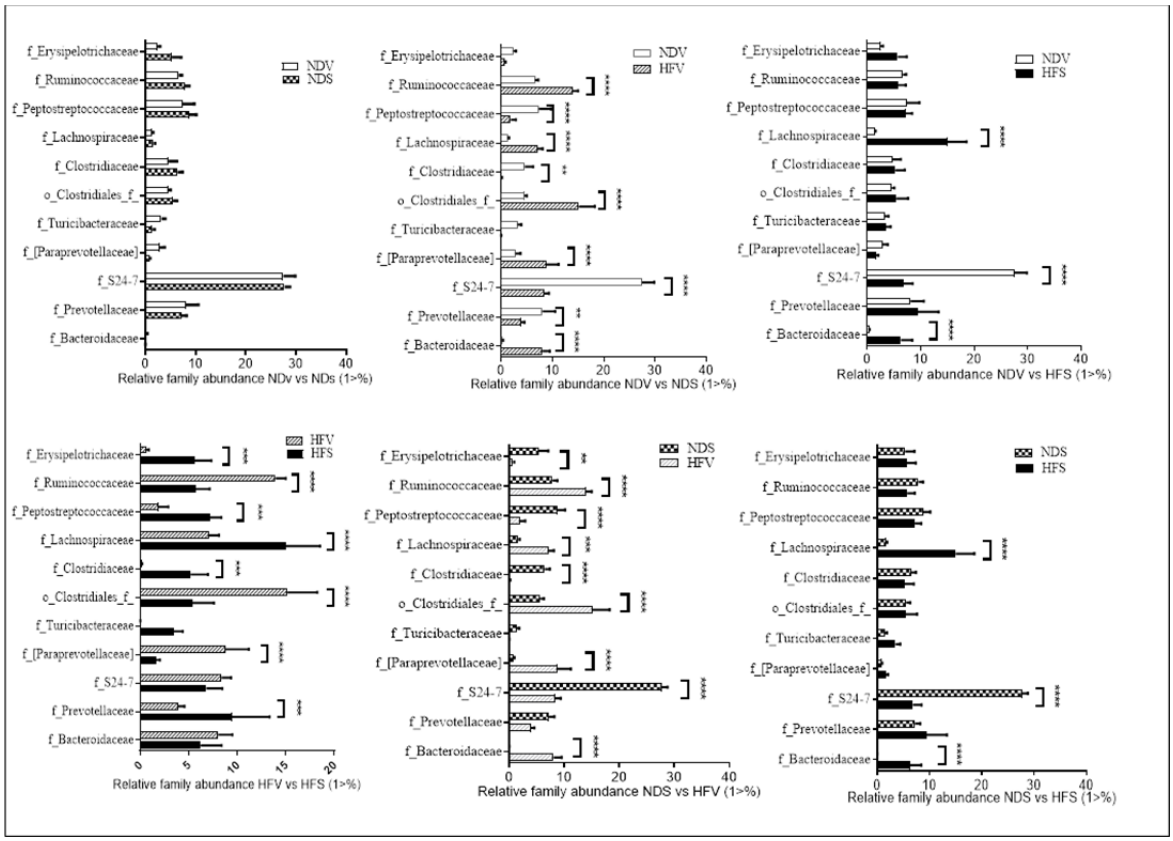


Figure S2: Bacterial taxonomy at family level



*P<0.05, ** P<0.01, ***P<0.001, ****P< 0.0001

Figure S3: Differences in abundance of families between the four experimental groups

The Bonferroni's comparison test was used to estimate the relationship of bacterial families between the groups (n=6/ group).

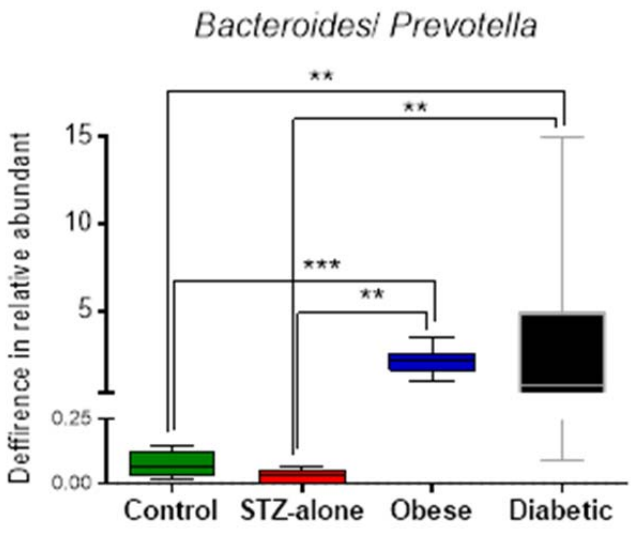
Table S3: Bacterial taxonomy at genera level

Phylum	Family	Genus	Control	STZ-alone	Obese	Diabetic
p_Actinobacteria	f_Bifidobacteriaceae	g_Bifidobacterium	0.00	0.00	0.01	0.00
	f_Coriobacteriaceae	f_Coriobacteriaceae_g__	0.14	0.13	0.14	0.14
		g_Adlercreutzia	0.14	0.13	0.14	0.00
		g_Atopobium	0.00	0.13	0.00	0.00
		g_Collinsella	0.00	0.00	0.00	0.14
p_Bacteroidetes	o_Bacteroidales_	o_Bacteroidales_g_	0.69	0.13	1.37	1.21
	f_Bacteroidaceae	g_Bacteroides	0.42	0.00	11.13	8.36
	f_Porphyromonadaceae	g_Parabacteroides	0.14	0.13	0.41	0.40
	f_Prevotellaceae	g_Prevotella	11.25	9.57	5.36	12.80
	f_RF16	f_RF16_g__	0.28	0.13	1.92	0.14
	f_Rikenellaceae	f_Rikenellaceae_g__	0.14	0.00	0.28	0.00
	f_S24-7	f_S24-7_g__	38.19	36.84	11.54	9.16
	f_[Odoribacteraceae]	g_Odoribacter	0.00	0.00	0.11	0.00
	f_[Paraprevotellaceae]	f_[Paraprevotellaceae]_g_	0.00	0.00	0.00	0.00
		g_CF231	0.83	0.27	2.47	0.67
		g_Paraprevotella	0.00	0.00	0.14	0.00
		g_YRC22	1.11	0.40	0.82	0.40
		g_[Prevotella]	1.94	0.53	8.65	1.08
p_Cyanobacteria	o_YS2	o_YS2_g__	0.14	0.00	0.41	0.00
p_Deferribacteres	f_Deferribacteraceae	g_Mucispirillum	0.00	0.00	0.00	0.14
p_Elusimicrobia	f_Elusimicrobiaceae	f_Elusimicrobiaceae_g__	0.00	0.00	0.00	0.00
p_Firmicutes	f_Lactobacillaceae	g_Lactobacillus	0.00	0.00	0.14	0.40
	f_Turicibacteraceae	g_Turicibacter	4.44	1.86	0.00	4.58
	o_Clostridiales_Other	o_Clostridiales_Other	0.00	0.27	0.00	0.14
	o_Clostridiales_	o_Clostridiales_g__	6.39	7.31	20.88	7.28
	f_Christensenellaceae	f_Christensenellaceae_g_	0.00	0.00	0.00	0.00
	f_Clostridiaceae	f_Clostridiaceae_g__	5.83	7.98	0.14	5.93
		g_Clostridium	0.56	0.67	0.00	1.08
		g_SMB53	0.14	0.00	0.00	0.00
	f_Lachnospiraceae	f_Lachnospiraceae_Other	0.00	0.00	0.28	0.14
		f_Lachnospiraceae_g__	0.97	1.06	2.34	2.29
		g_Anaerostipes	0.14	0.13	0.00	0.00
		g_Blautia	0.14	0.13	1.65	10.65
		g_Coprococcus	0.28	0.67	1.65	1.48
		g_Dorea	0.14	0.13	0.96	2.16
		g_Lachnobacterium	0.00	0.00	0.00	0.00
		g_Roseburia	0.00	0.00	1.37	2.70
		g_[Ruminococcus]	0.14	0.13	1.65	0.81
	f_Peptostreptococcaceae	f_Peptostreptococcaceae_g	10.28	11.70	2.61	9.70
	f_Ruminococcaceae	f_Ruminococcaceae_Other	0.00	0.00	0.00	0.00
		f_Ruminococcaceae_g__	4.17	3.86	4.53	2.56

		g_Oscillospira	1.53	2.66	7.69	2.97
		g_Ruminococcus	3.47	3.86	6.87	2.29
	f_[Mogibacteriaceae]	f_[Mogibacteriaceae]_Other	0.00	0.00	0.00	0.00
		f_[Mogibacteriaceae]_g__	0.14	0.27	0.28	0.14
	f_Erysipelotrichaceae	f_Erysipelotrichaceae_g__	0.14	0.00	0.14	0.54
		g_Allobaculum	3.19	6.92	0.69	6.74
		g_Coprobacillus	0.00	0.00	0.00	0.00
		g_p-75-a5	0.00	0.13	0.00	0.00
		g_[Eubacterium]	0.00	0.00	0.00	0.27
p_Fusobacteria	f_Fusobacteriaceae	g_Fusobacterium	0.00	0.00	0.00	0.00
p_Proteobacteria	c_Alphaproteobacteria_g	c_Alphaproteobacteria_g_	0.00	0.00	0.00	0.00
	o_RF32_g__	o_RF32_g__	0.14	0.00	0.28	0.00
	f_Alcaligenaceae	g_Sutterella	0.00	0.00	0.00	0.00
	f_Desulfovibrionaceae	f_Desulfovibrionaceae_g_	0.00	0.00	0.14	0.14
		g_Desulfovibrio	0.00	0.13	0.00	0.00
	f_Helicobacteraceae	f__Helicobacteraceae_g_	0.00	0.00	0.00	0.00
p_Spirochaetes	f_Spirochaetaceae	g_Treponema	0.00	0.00	0.55	0.40
p_TM7	f_F16	f_F16_g__	0.14	0.13	0.00	0.00
p_Tenericutes	o_RF39	o_RF39_g__	2.22	1.60	0.41	0.00

The Phylogenetic analysis at genus level of faecal 16S rRNA for the four experimental groups (n=6/ group). The mean abundance (%) from the bacterial taxonomic profiling is shown. Values <0.01% show as 0.00 due to decimal point truncation.

Key Control= normal diet; STZ-alone= normal diet plus STZ injection; Obese= high fat diet and Diabetic= high fat diet plus STZ injection.



** P<0.01, ***P<0.001

Figure S4: Ratio of *Bacteroides/ Prevotella* among the four groups

Table S4: Differences of bacterial beta diversity: unweighted (a) and weighted (b) in the individual animal samples

a unweighted

	NDV1	NDV2	NDV3	NDV4	NDV5	NDV6
NDV1	0	0.08	0.11	0.11	0.08	0.09
NDV2	0.08	0	0.08	0.13	0.11	0.12
NDV3	0.11	0.08	0	0.14	0.13	0.14
NDV4	0.11	0.13	0.14	0	0.11	0.13
NDV5	0.08	0.11	0.13	0.11	0	0.07
NDV6	0.09	0.12	0.14	0.13	0.07	0

	NDS1	NDS2	NDS3	NDS4	NDS5	NDS6
NDS1	0	0.09	0.08	0.13	0.07	0.09
NDS2	0.09	0	0.13	0.16	0.12	0.1
NDS3	0.08	0.13	0	0.11	0.06	0.09
NDS4	0.13	0.16	0.11	0	0.1	0.12
NDS5	0.07	0.12	0.06	0.1	0	0.08
NDS6	0.09	0.1	0.09	0.12	0.08	0.0

	HFV1	HFV2	HFV3	HFV4	HFV5	HFV6
HFV1	0	0.09	0.07	0.09	0.1	0.1
HFV2	0.09	0	0.09	0.07	0.11	0.08
HFV3	0.07	0.09	0	0.07	0.13	0.09
HFV4	0.09	0.07	0.07	0	0.11	0.07
HFV5	0.1	0.11	0.13	0.11	0	0.12
HFV6	0.1	0.08	0.09	0.07	0.12	0

	HFS2	HFS3	HFS4	HFS5	HFS6	HFS1
HFS2	0.00	0.15	0.15	0.14	0.16	0.17
HFS3	0.15	0.00	0.14	0.17	0.18	0.08
HFS4	0.15	0.14	0.00	0.11	0.12	0.18
HFS5	0.14	0.17	0.11	0.00	0.14	0.21
HFS6	0.16	0.18	0.12	0.14	0.00	0.22
HFS1	0.17	0.08	0.18	0.21	0.22	0.00

b weighted

	NDV1	NDV2	NDV3	NDV4	NDV5	NDV6
NDV1	0	0.22	0.19	0.08	0.1	0.09
NDV2	0.22	0	0.05	0.25	0.17	0.27
NDV3	0.19	0.05	0	0.21	0.15	0.25
NDV4	0.08	0.25	0.21	0	0.12	0.11
NDV5	0.1	0.17	0.15	0.12	0	0.14
NDV6	0.09	0.27	0.25	0.11	0.14	0

	NDS1	NDS2	NDS3	NDS4	NDS5	NDS6
NDS1	0	0.1	0.1	0.18	0.07	0.09
NDS2	0.1	0	0.1	0.14	0.08	0.08
NDS3	0.1	0.1	0	0.21	0.09	0.11
NDS4	0.18	0.14	0.21	0	0.18	0.14
NDS5	0.07	0.08	0.09	0.18	0	0.08
NDS6	0.09	0.08	0.11	0.14	008	0.1

	HFV1	HFV2	HFV3	HFV4	HFV5	HFV6
HFV1	0	0.11	0.03	0.14	0.16	0.08
HFV2	0.11	0	0.12	0.11	0.09	0.08
HFV3	0.03	0.12	0	0.15	0.18	0.09
HFV4	0.14	0.11	0.15	0	0.09	0.1
HFV5	0.16	0.09	0.18	0.09	0	0.13
HFV6	0.08	0.08	0.09	0.1	0.13	0

	HFS2	HFS3	HFS4	HFS5	HFS6	HFS1
HFS2	0.00	0.10	0.24	0.17	0.23	0.14
HFS3	0.10	0.00	0.19	0.12	0.16	0.14
HFS4	0.24	0.19	0.00	0.13	0.13	0.18
HFS5	0.17	0.12	0.13	0.00	0.12	0.15
HFS6	0.23	0.16	0.13	0.12	0.00	0.22
HFS1	0.14	0.14	0.18	0.15	0.22	0.00

Key: NDV = normal diet; NDS = normal diet plus STZ injection; HFV = high fat diet (Obese); HFS = high fat diet plus STZ injection (Diabetic).

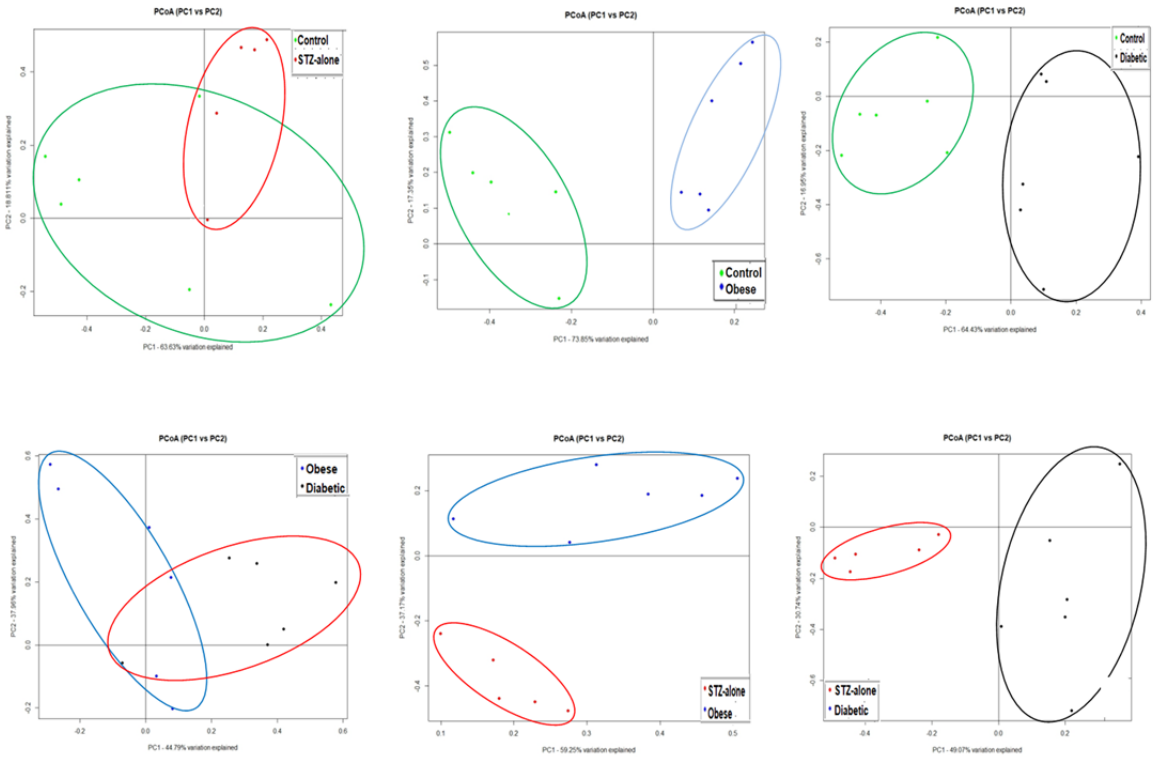


Figure S5: Bacterial β-diversity (weighted UniFrac) of the microbial communities

The bacterial communities of individual rats based on the weighted UniFrac by PCoA.

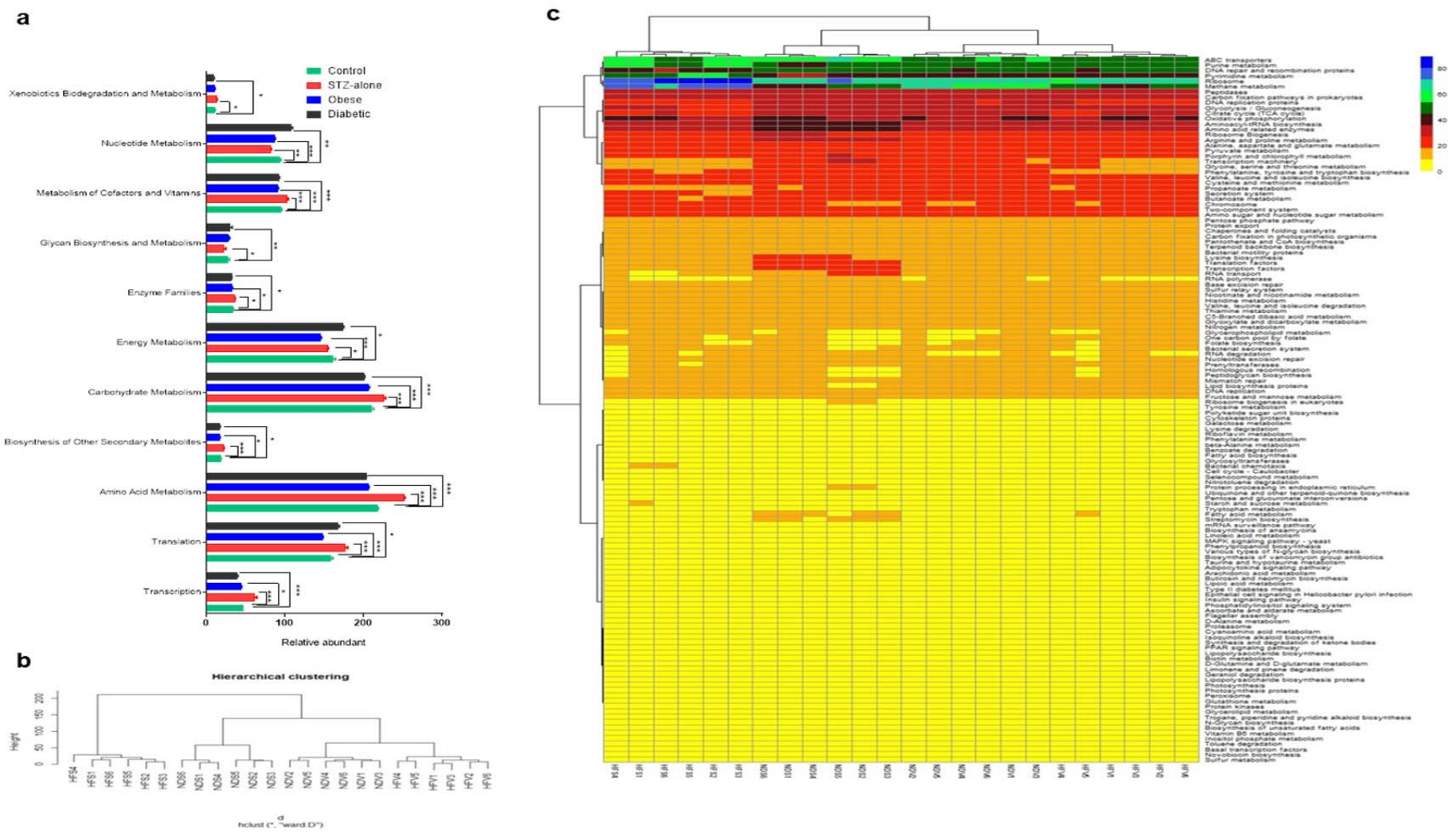
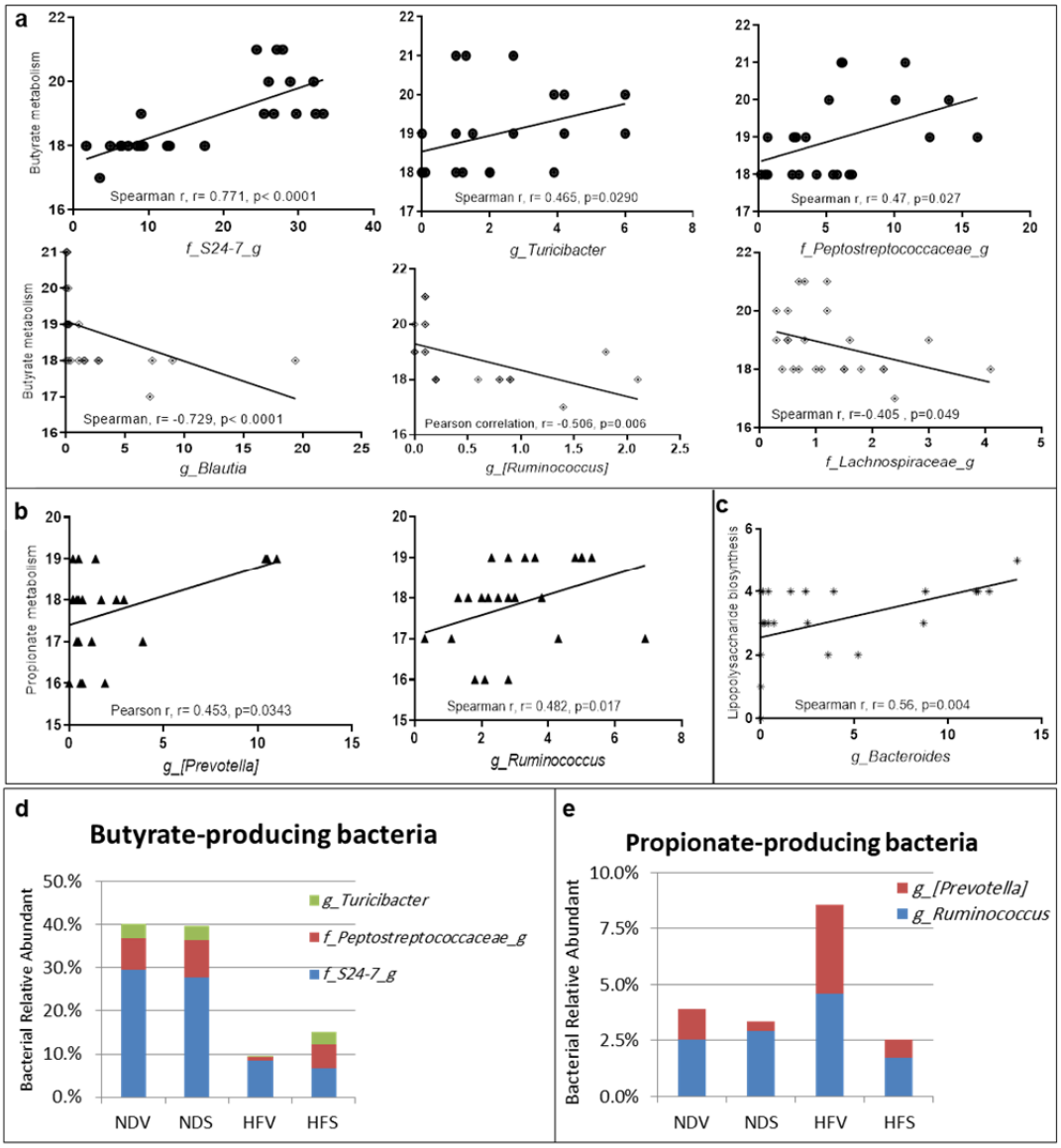


Figure S6: PICRUSt analysis of microbial communities at level 2 (a) and level 3; Hierarchical clustering (b) and heatmap (c)

Hierarchical clustering and Heat map analysis by R statistic (n=6). The Hierarchical clustering was used to confirm the similarity of bacterial function between the groups. Heatmap was use to illustrate the relative abundant of each individual bacterial function among these four groups. Key NDV = normal diet (Control); NDS = normal diet plus STZ injection (STZ-alone); HFV = high fat diet (Obese); HFS = high fat diet plus STZ injection (Diabetic).



*P<0.05

Figure S7: Correlations between bacteria and metabolic activities

Linear correlation Pearson coefficients and nonparametric Spearman were used to show the relationship of gut bacteria either with SCFAs (butyric acid (a), propionic acid (b)) and LPS biosynthesis (c) and the relative abundance of bacteria that produce butyric acid (d) and propionic acid (e).